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Wound Healing

Edited by

WILLIAM MONTAGNA

RUPERT E. BILLINGHAM

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Wound Healing

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PREFACE

EACH chapter in this book was presented to the 13th annual symposium on the Biology of Skin at Brown University, on 26 and 27 January 1963. This is a milestone in the history of these symposia since it marks the last one to be held at Brown University. Beginning in 1964, the symposia will be conducted at the University of Oregon Medical School in Portland, Oregon, and the series of the *Advances in Biology of Skin* will continue uninterrupted.

As all of the previous symposia, this one was aided by financial gifts from Abbott Laboratories, American Cholesterol Products, Inc., Burroughs Wellcome & Co., Inc., Chesebrough–Pond's, Inc., Colgate–Palmolive Co., Dome Chemicals, Inc., E. I. duPont deNemours & Co., The Gillette Co., Johnson & Johnson, The Kendall Co., Lever Brothers Co., Procter & Gamble Co., Revlon, Inc., Schering Foundation, The Squibb Institute for Medical Research, The Upjohn Co., Warner–Lambert Research Institute, and Westwood Pharmaceuticals. We thank these agencies for their continued generosity. We express our special thanks to Brown University, for being a matchless host institution. Brown University has made valuable contributions to biology and to medicine in encouraging the continuation of these symposia.

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INTRODUCTION

To MOST of us, the mere mention of "wound healing" evokes a mental image of some sort of cutaneous lesion. This is natural since skin and the cornea (a very closely related variant) are the only tissues that we can normally see, and the only tissues exposed to the common hazards of everyday life. Furthermore, for obvious reasons, skin wounds are the lesions of choice for experimental study, as evidenced by the contents of this book. We should, however, bear in mind that not all of the principles that apply to the healing of skin wounds are necessarily of *general* application; some of these are probably specific to the skin. We must not overlook the existence of other tissues and organs, some of which, such as bones, also possess remarkable reparative potentials. In mammals, the liver is at least one tissue with an even superior facility for self-repair than skin. Why Nature should have bestowed this apparently unnecessary blessing upon such a well-protected organ has yet to be explained. One may speculate, that in spite of its sheltered position, the liver does not escape damage from chemical or microbial agents.

Wound healing must surely be one of the oldest common meeting-grounds of experimental biology and medicine. Yet, until fairly recently, progress amounted to little more than rationalizations of empirically evolved surgical procedures. Reflecting upon this state of affairs, Dr. Dunphy recently stated that "two centuries of inquiry into the nature of the healing process has brought us but a little way beyond John Hunter. . . ." We now feel less pessimistic than Dr. Dunphy about our understanding of wound healing. The problem, though still in the descriptive stage, is undergoing a great re-invigoration and is being placed upon a sound experimental basis through the introduction and timely application of quantitative methods of analysis by Carrel, Howes, Abercrombie, Dunphy and others.

It would be a grave mistake to regard the principal objective of wound healing as a discipline *sui generis*, to provide medicine with improved means of dealing with wounds, or even more optimistically, to try and promote super-normal healing, or spectacular regenerations. No one has suggested that the proper goal of embryology is to devise means of growing better men, or of growing them more rapidly. To arrive at an understanding of the nature, and control of the closely integrated processes that lead to the natural repair of many types of wounds is a perfectly adequate justification for our endeavors.

Many of these processes go on in the body normally, unassociated with wound healing.

Among the more important unsolved problems and topics which can be grouped under the general heading of wound healing are: the origin of the fibroblastic cell population of a wound, and its ultimate fate; the dynamics and interrelationships of the cell populations of wounds; the role of controlling mechanisms that regulate cell replacement or turnover activity in normal tissues and in wound healing; the principles that govern the movement of epithelial sheets over denuded areas; the mechanism of wound contracture and, finally, causal analyses of rare but grandiose regenerative phenomena such as the growth and replacement of antlers in certain mammals. These and other problems are presented in this book. Some important considerations, such as the influence of systemic factors, have had to be omitted from the symposium, because of time limitation, and from this book, lest the volume become too large.

To keep us humble and on a frivolous note, we suggest that surgeons will never be able to compete with the farrier who successfully sewed back into place, with the aid of fresh sprigs and laurel shoots, the entire rear portion of the Lithuanian horse which the intrepid Baron Boris Von Munchausen had just ridden under a falling portcullis.

R. E. BILLINGHAM
W. MONTAGNA

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CHAPTER I

BIOLOGICAL CONSIDERATIONS OF WOUND HEALING

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I. INTRODUCTION

BY BIOLOGICAL considerations is meant the study of wound healing in relation to the survival of individual organisms and of their species—the evolutionary aspects of the process in fact. Biological aspects may be set in antithesis to physiological aspects, the strategy as contrasted with the tactics, the "Why?" as distinct from the "How?" The antithesis is effectively that between the *final*, or ultimate, and the *efficient*, or proximate, causes of Aristotle—though to him the final cause was perhaps something more supernatural than we suppose today. Of course, it is not so very certain, when we claim that the final causes of any biological phenomenon are evolutionary forces, that we have effected a great revolution against the Aristotelian view, since strictly speaking we know virtually nothing of the actual causes of evolution, but only that Natural Selection sits in judgment on their efforts. However, final causation, as we now understand it, is at least open to observation and experiment and this is a very real advance. We can try to find out how often individuals of the different species are wounded in the field, how closely this frequency is correlated with their power of healing, to what extent rapid healing contributes to the survival of individuals and their species, how far healing has improved or deteriorated during evolution, what biological considerations are paramount in all organs which heal particularly well, or very badly, and so on.

It is, of course, possible to study wound healing with great profit solely from the physiological aspect, without having to worry about final causes, but no doubt any knowledge of the latter is likely to deepen our understanding of the process, and to prompt new lines of research into the proximate causes. More specifically, final causes must work through proximate mechanisms and so should help in analyzing the latter. The exact nexus between final and efficient causation in regeneration has hardly begun to attract specific attention (Spilsbury, 1961). It is primarily a genetic–morphogenetic problem and has long been a centre of interest in the study of embryogenesis.

Although the interest of this symposium is primarily in skin, the healing of other tissues is relevant since much the same final causes operate in all. Equally, regeneration in the lower animals is as relevant as wound healing in mammals for there is probably no hard and fast distinction between them (Russell and Billingham, 1962). They are merely extremes of a continuous spectrum of powers of repair. We see something like a complete spectrum within the mammals alone: they regenerate only the peripheral processes of their nerve cells, but they repair whole tissue-layers in the skin, more massive, highly organized structures in bone-healing, and finally some can replace as much as two-thirds of the liver. Wound healing, therefore, can be fully understood only in its setting within the complete spectrum.

There is need to defend this thesis since it is possible to bring forward evidence that wound healing is significantly different from regeneration (Millot, 1931). The hydrozoan, *Tubularia*, sometimes regenerates lost hydranths while at other times it merely closes the wound. However, wound-healing in mammals is not such a simple process as this. Locally it involves the same sequence of events as in typical acts of regeneration, falling into two main periods, a regressive and a progressive repair phase. Systemically, both involve the same *stress* syndrome (Needham, 1955), consisting of the oscillatory sequence: shock, post shock depression (ebb), alarm reaction (flow), and general adaptation syndrome (Selye, 1948, 1955; Cuthbertson, 1954). This syndrome does not occur in the normal growth processes of the skin, the so-called *physiological regeneration*, or in other types of morphogenesis. It is not restricted to traumatic damage, however.

II. ADAPTIVE CHARACTER OF REGENERATION

In most cases there is scarcely any doubt that wound healing and regeneration play a useful part in the life and survival of animals and it is only in fairly recent years that this has been seriously questioned. We are all very conscious of the inconvenience, or worse, of even the most trivial of wounds, and if this did not heal, the strain would be continuous, increasing and accelerating, owing to infection and other secondary complications. If we estimate that there is an average of one new minor wound a week, a conservative figure for some of us, this would amount to 3500 or more in a lifetime of 70 years—except that few individuals would be able to boast such a life span under those conditions. If such a hypothetical situation did in fact exist, then, no doubt, there would be fatal stress, loss of body fluid, or infection, long before the end of the effective reproductive age, and this is the really critical consideration for species-survival (Medawar, 1946). Healing is a major concern of physicians and surgeons, even under the relatively favourable conditions which actually exist.

In the field, as many as 93 per cent of the individuals of some species may be found to have lost major organs or parts of the body (Needham, 1953), and there seems little doubt that the power to regenerate the losses must have survival value in these cases. Knowing the average rate of regeneration in a particular species and the incidence of detectable losses in process of regeneration at any time, it is possible to estimate how many times the average individual incurs a major loss before the end of its reproductive life. In triclad planarians, which continue to reproduce virtually to the end of their lives, there must be three or four such losses (Needham, 1961), and prompt regeneration must have considerable survival value for the species.

It seems reasonably certain, therefore, that the ability to heal wounds is a biological adaptation and has been subjected to natural selection. The alternative hypothesis that it is merely a casually persisting pristine power of morphogenesis does not bear examination. On the one hand, it is doubtful if any property persists merely casually, and on the other it seems likely that this power must have been as useful and adaptive to the earliest discrete organisms as it is now. Even before the days of discrete unicellular organisms there was, no doubt, a process of molecular replacement—which still persists as one component of *metabolic turnover*. It is a profitable point for speculation whether the gross regeneration in which we are here mainly interested could have evolved continuously and spontaneously from a molecular replacement mechanism of this kind.

More apposite is the question whether wound healing and regeneration today show any improvement compared with the pristine processes, i.e. any evidence for progressive evolution. In many cases, the answer is "yes". Many animals have evolved ingenious autotomy-mechanisms (Korschelt, 1927) to facilitate the amputation of parts seized or damaged (Fig. 1)—provided the parts are temporarily dispensable. The wound is closed by special devices, sphincter muscles in annelids and other animals with soft bodies, and a preformed partition across the stump of the crustacean limb (Fig. 1, B). Until autotomy the crustacean partition must have holes for the nerve and blood vessels of the limb, but these are closed immediately after the act.

The lizard's tail and some other autotomized organs continue to writhe after autotomy and so distract the attention of a predator—and even of a forewarned human being. This must be an important contributory factor to the survival of the animal itself. Regeneration is more rapid following amputation at the autotomy plane than at any other level, proximally or distally to it. In all its features, the mechanism is highly perfected. It is worth noting, in passing, that autotomy can be a device of survival value even if it is not followed by regeneration, and certain insects and chelicerates seem to have perfected it under the selection pressure of escape alone. Adult tipulids (crane flies) were found to autotomize legs in 59 per cent of cases, even when captured as carefully as possible, and 5 per cent had long standing losses, with a dark scab over the wound. Like most adult insects they cannot regenerate their legs (see p.18). This situation is a salutory warning against assuming too much similarity between the mechanisms of different animals, or that all components of the regeneration mechanism are necessarily rigidly linked.

Reciprocally, there are some organs which will not autotomize but nevertheless regenerate well if amputated. The fifth pair of thoracic legs of the male,

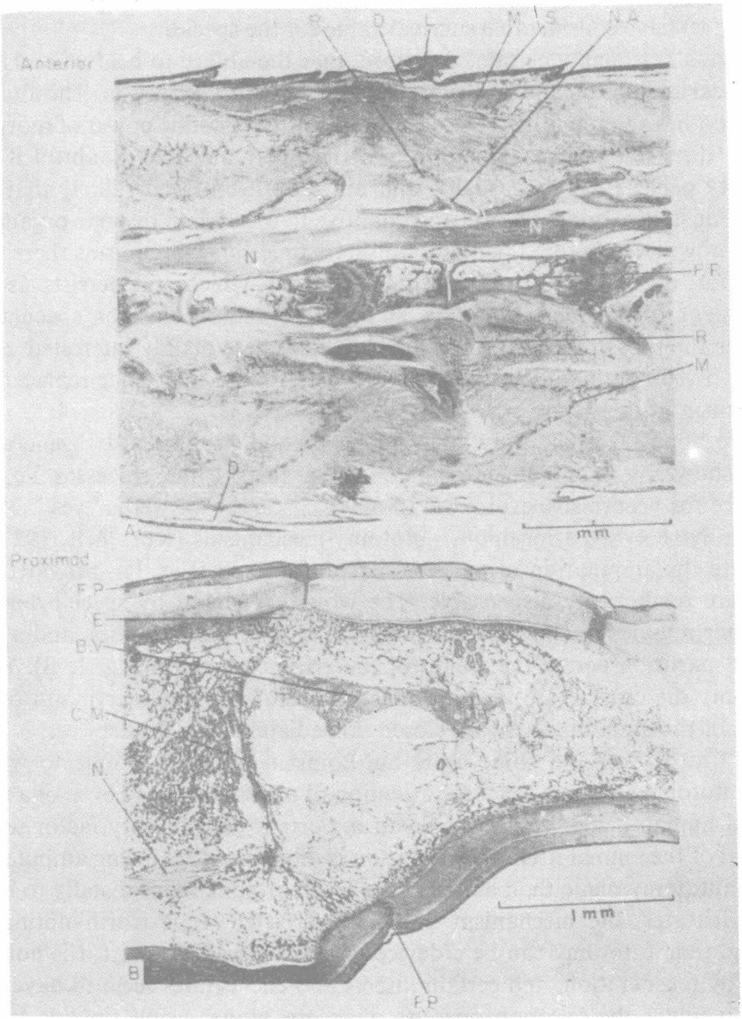

FIG. 1

in the isopod crustacean *Asellus*, is an example. During the breeding season this pair of limbs, which is used to hold the female during coition, will not autotomize, though it regenerates after amputation as well as it does in the juvenile stage—when it will also autotomize (Needham, 1942). This is true also of the last pair of abdominal legs of the hermit crab, the uropods, which are essential to the safety of the animal and were never found to be lost in the field (Morgan, 1898; Needham, 1961) but which regenerate well if amputated. Morgan used this as evidence that regenerative power is not always proportional to the need for it, since these limbs are well protected inside the adopted shell of the hermit. However, it seems likely that regenerative power is actually related to the indispensability of the organ, whereas readiness to autotomize is inversely related to this. In the female crab the other abdominal limbs also have an important function, to carry fertilized eggs, and they do not autotomize, as they do in the male (Needham, 1961). Structures which both autotomize and regenerate are in a compromise situation, relatively important but more dispensable than the whole animal. Incidence of loss in the field is not purely accidental and is not an ideal measure of the need for regeneration.

In many cases the processes of wound-healing are so rapid and efficient that there can be no doubt that they are the result of a long history of selective improvement. The primary and secondary closure of wounds, destruction of foreign organisms, demolition of damaged cells, dedifferentiation of tissues to mobilize cells, the migration and proliferation of these and their subsequent

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FIG. 1A (*opposite*)

Sagittal section through part of the tail of the lizard, *Lacerta viridis*, to show the provision for autotomy. The fracture planes (F.P.) across the middle of the centrum of each vertebra, continue through the neural arch (N.A.) above, and then follow in order (1) a cleft in the connective tissue, (2) the myocommata (M) between muscle somites and (3) a line (D) through the dense connective tissue of the dermis, to connect with (4) the loose connective tissue (L) between alternate rings of scales (S). There are two rings of scales per vertebra. Ventrally the fracture plane is continued by a cleft through thick reticulate connective tissue (R) and then follows the myocommata, etc., as above. The line (D) through the dermis ventrally is not very clear on the print. There is no obvious provision in the nerve cord (N). Photo, J. S. Haywood.

FIG. 1B (*opposite*)

Longitudinal section through the basal part of the leg of a crayfish, *Potamobius* (*Astacus*) *fluviatilis*, to show the provision for autotomy. There is a fracture plane (F.P.) through the exoskeleton on both sides but the tissues inside tear easily and need no provision. A connective tissue membrane (C.M.) seals the stump after fracture, the aperture for the blood vessels (B.V.) being sealed by a blood clot. The nerve (N) to the leg is seen passing through a cuff in the membrane. The epidermis (E) at the fracture plane differs from that elsewhere. Photo, J. S. Haywood.